Acute Respiratory Distress Syndrome Phenotypes

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Abstract

The acute respiratory distress syndrome (ARDS) phenotype was first described over 50 years ago and since that time significant progress has been made in understanding the biologic processes underlying the syndrome. Despite this improved understanding, no pharmacologic therapies aimed at the underlying biology have been proven effective in ARDS. Increasingly, ARDS has been recognized as a heterogeneous syndrome characterized by subphenotypes with distinct clinical, radiographic, and biologic differences, distinct outcomes, and potentially distinct responses to therapy. The Berlin Definition of ARDS specifies three severity classifications: mild, moderate, and severe based on the PaO_2 to FiO_2 ratio. Two randomized controlled trials have demonstrated a potential benefit to prone positioning and neuromuscular blockade in moderate to severe phenotypes of ARDS only. Precipitating risk factor, direct versus indirect lung injury, and timing of ARDS onset can determine other clinical phenotypes of ARDS after admission. Radiographic phenotypes of ARDS have been described based on a diffuse versus focal pattern of infiltrates on chest imaging. Finally and most promisingly, biologic subphenotypes or endotypes have increasingly been identified using plasma biomarkers, genetics, and unbiased approaches such as latent class analysis. The potential of precision medicine lies in identifying novel therapeutics aimed at ARDS biology and the subpopulation within ARDS most likely to respond. In this review, we discuss the challenges and approaches to subphenotype ARDS into clinical, radiologic, severity, and biologic phenotypes with an eye toward the future of precision medicine in critical care.

Keywords

- acute respiratory distress syndrome
- acute lung injury
- phenotype
- precision medicine

Despite earlier descriptions of noncardiogenic pulmonary edema, the phenotype we now refer to as the acute respiratory distress syndrome (ARDS) was first described in a case series published in the *Lancet* just over 50 years ago.[1] In this study, Ashbaugh and colleagues describe 12 adults with acute onset hypoxic respiratory failure and poor lung compliance. The patients demonstrated similar pathophysiology despite distinct insults ranging from trauma to pneumonia to pancreatitis. The authors also described improvements in oxygenation with the application of positive end expiratory pressure (PEEP), an observation that revolutionized early ARDS care. Since this initial phenotypic description, multiple clinical definitions have been proposed and evolved as our understanding of ARDS has improved.[2–5] Currently, the 2012 Berlin Definition of ARDS defines the syndrome as the acute onset of hypoxia and bilateral pulmonary opacities not fully explained by a cardiac cause.[4] Acute onset is specified to be within 1 week of a precipitating illness and hypoxia is determined by a PaO_2 to FiO_2 ratio less than or equal to 300 mm Hg while receiving a minimum of 5 cm H_2O of PEEP.

The development of broad consensus definitions of ARDS, including the previous American–European Consensus Definition,[3,4] has allowed for the completion of clinical trials demonstrating a therapeutic benefit to several supportive care interventions, including lung protective mechanical ventilation and prone positioning.[6,7] Unfortunately, however, the

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large majority of ARDS clinical trials have failed, particularly trials of pharmaceutical interventions targeting ARDS biology.[8] The largest challenge in phenotyping ARDS is the lack of a simple diagnostic test, resulting in the reliance on a consensus definition developed by experts.[4,9] The current consensus definition of ARDS remains challenging, as chest radiograph interpretation has poor inter-rater reliability and clinicians routinely fail to recognize ARDS when treating patients.[10–14] Additionally, given the current intentionally broad definitions of ARDS, the syndrome has marked clinical, radiologic, pathologic, and biologic heterogeneity. In an autopsy study of patients who died with ARDS, only 45% demonstrated the histopathologic correlate of ARDS, diffuse alveolar damage (DAD), with the other 55% percent demonstrating a variety of other pathologic findings.[15] It is this heterogeneity that is hypothesized to underlie many failures in translation of promising preclinical therapeutics to patient populations. In this review, we aim to outline the current approaches to understanding ARDS heterogeneity by identifying subphenotypes of ARDS with distinct clinical, radiologic, or biologic characteristics. We will discuss how unpacking heterogeneity has led to some early successes, and the potential for future success with this approach with an eye toward personalized medicine in ARDS care.

Evolution of Phenotypes, Subphenotypes, and Endotypes

A phenotype is defined as the set of observable characteristics or traits of an organism resulting from the interaction of genotype and the environment. The term has often been used to describe syndromes and disorders in medicine believed to develop from this interaction. Naturally, the identification of a phenotype in medicine begins with a description of a group of individuals displaying similar characteristics, not dissimilar to how Ashbaugh and colleagues initially described 12 cases of ARDS.[1] As a phenotype is investigated further, a natural evolution occurs whereby the phenotype is better characterized. By better characterizing a phenotype, misclassification of similar phenotypes is reduced and subtypes are discerned based on unique biology, clinical characteristics, or response to treatment. In cardiology, the acute coronary syndrome was initially described as sudden onset chest pain that often resulted in death.[16] This initial syndrome is now commonly differentiated into unstable angina, non-ST elevation myocardial infarction, and ST elevation myocardial infarction based on electrocardiogram and troponin measurements. These three subtypes of the acute coronary syndrome reflect similar but distinct biology and call for distinct interventions.

Inherent in the process of syndrome evolution is the understanding of endotypes, or subtypes of a syndrome defined by distinct biology.[17] In pulmonary medicine, the understanding of asthma has evolved from one disorder to multiple endotypes with different clinical presentations, prognoses, and responses to therapy. Initially described as a chronic disorder of the lung characterized by variable airflow obstruction and an underlying inflammatory process, asthma has long been recognized as heterogeneous.[18] Early descriptions of asthma divided patients into two subgroups, "extrinsic" or atopic asthma caused by inhalation of allergens and "intrinsic" or nonatopic asthma.[19] As the biologic understanding of asthma improved, two distinct inflammatory endotypes of asthma based on the presence or absence of eosinophils were described.[20] More recently, unbiased clustering algorithms incorporating clinical and biological variables have identified multiple clinical phenotypes of asthma.[21–23] Additionally, therapies that target specific inflammatory mediators based on biomarker-defined endotypes are now in use in asthma, including the anti-immunoglobulin E therapeutic omalizumab.[24] Similar phenotype evolutions can be described for chronic obstructive pulmonary disease,[25] pulmonary vasculitis,[26] and interstitial lung disease.[27]

Outside of pulmonary medicine, the field of oncology has had tremendous success in therapeutically targeting endotypes defined by specific cancerous mutations. For example, the first step toward precision medicine in lung cancer was the development of chemotherapy regimens based on cancer histology and stage.[28] With the advent of mutation-targeted therapies, lung cancer therapeutics have now progressed to precision medicine based on tumor molecular profiles. Specifically, epidermal growth factor receptor (EGFR) mutations predict response to EGFR tyrosine kinase inhibitors gefitinib, erlotinib, and afatinib,[29,30] and anaplastic lymphoma kinase (ALK) mutations predict response to crizotinib.[31] In earlier trials of unselected non-small cell lung cancer patients, some of these same therapeutics either failed to demonstrate a benefit or only demonstrated a marginal benefit.[32,33] It was the recognition of biologically defined endotypes that resulted in the identification of subgroups of patients most likely to therapeutically respond. Similar molecular-targeted therapeutics have been developed in breast cancer, colorectal cancer, melanoma, and others.[34–36]

While there is a significant amount to learn from the success of subtyping asthma and cancer, the ARDS phenotype has unique challenges that make this task difficult. First, the acute and critical nature of ARDS makes subtyping or endotyping ARDS extremely time sensitive to provide a targeted therapeutic early in the syndrome. If biomarkers are used to subtype ARDS, they must be easily and rapidly measured with high reproducibility and validity. Second, pathology via biopsy is not commonly obtained in ARDS due to the risks and therefore will not likely routinely be used for ARDS phenotype identification as it is in cancer. Third, clinicians frequently fail to recognize ARDS, which is a necessary first step in subtype identification. Despite these challenges, significant recent progress has been made in phenotyping ARDS (►**Table 1**), and researchers are consistently moving toward understanding biologically defined endotypes of the syndrome. There is substantial overlap between the terms phenotype, subphenotype, and endotype, and a subphenotype initially defined by a clinical or radiographic factor may subsequently be found to identify unique biology.

Severity Phenotypes of ARDS

The Berlin Definition of ARDS proposed three severity phenotypes; mild, moderate, and severe, defined by the ratio of

Table 1 Phenotypes of acute respiratory distress syndrome

Phenotype	Description	Differences	Potential therapies	References
Hypoxia severity phenotypes	Berlin categories: Mild: $200 < PaO_2/FiO_2 < 300$ Mod: $100 < PaO_2/FiO_2 < 200$ Severe: $PaO_2/FiO_2 < 100$	• Severity of hypoxia • DAD more likely pathology in severe	• Prone positioning ($PaO_2/FiO_2 < 150$) • Cisatracurium ($PaO_2/FiO_2 < 150$)	4,7,41
ARDS by precipitating risk factor	Precipitating factors including: sepsis, trauma, pneumonia, aspiration, transfusion, pancreatitis	• Differences in ARDS risk, severity, and mortality		53,56–60
Direct versus indirect lung injury	Direct: pneumonia, pulmonary contusion, aspiration Indirect: nonpulmonary sepsis, nonthoracic trauma, transfusions	• Epithelial vs. endothelial injury • Differences in mortality	• Epithelial vs. endothelial targeted therapies • Indirect more likely to respond to PEEP	61,64–74
Timing of onset phenotypes	Early onset developing <48 h from admission versus late onset >48 h from admission	• Different clinical characteristics • Elevated RAGE and Ang-2 in early onset		75–78
Radiographic phenotypes	Nonfocal/diffuse vs. focal/lobar on chest imaging	• Differences in morality, lung compliance, indirect lung injury, and plasma RAGE level	• Diffuse more likely to respond to PEEP	81–86
Genetic defined endotypes	Endotypes of ARDS defined by genetic variability that alters ARDS risk, outcome, or response to treatment	• Distinct ARDS risk, outcome, or response to treatment	• Therapies targeting biology implicated by genetic variants	88,89
Biomarker defined endotypes	Endotypes of ARDS defined by biomarker measurements	• Distinct ARDS risk, outcome, or response to treatment	• Therapies targeting biology implicated by biomarker elevation	109–119,124 130,131
Hyperinflammatory versus uninflamed	Endotypes of ARDS determined from unbiased latent class analysis and cluster analysis	• Hyperinflammatory characterized by elevated plasma inflammatory biomarkers, and higher mortality	• Phenotypes responded differently to PEEP and fluid strategy • Survival benefit observed in response to simvastatin in hyperinflammatory phenotype	142–144,147 148,151,152

Abbreviations: Ang-2, angiopoietin-2; ARDS, acute respiratory distress syndrome; DAD, diffuse alveolar damage; PEEP, positive end expiratory pressure; RAGE, receptor for advanced glycation end-products.

PaO_2 to FiO_2.[4] When applied to multicenter clinical datasets, the higher severity stages were associated with a higher mortality, ranging from 27% for mild, 32% for moderate, and 45% for severe ARDS. While targeting the PaO_2/FiO_2 ratio as a surrogate outcome for mortality has largely failed in clinical trials, the Berlin severity categories have the potential to serve for prognostic enrichment.[37,38] Prognostic enrichment is a strategy whereby a clinical trial enrolls patients more likely to experience an outcome of interest, reducing the sample size needed to detect a treatment effect of the intervention.[39] In addition to predicting mortality, severity categories based on the PaO_2/FiO_2 ratio may also help identify distinct subtypes of disease. In the previously discussed autopsy study of 356 patients with ARDS, DAD was only present in 45%; however, those patients with moderate and severe ARDS were significantly more likely to have DAD on pathology.[15] This finding suggests that the moderate and severe subtypes identify a more homogeneous population with ARDS. Therefore, the moderate–severe Berlin severity categories may also serve for predictive enrichment. Predictive enrichment is a strategy by which patients more likely to respond to a specific therapy are enrolled in a clinical trial.[40] If an intervention in ARDS is aimed at treating the biology that results in DAD, it may be more likely to have a demonstrable benefit in the more homogeneous moderate to severe subgroups of ARDS.

An approach targeting a severe phenotype of ARDS based on the PaO_2/FiO_2 ratio has been successful in two randomized controlled trials, the ARDS et Curarisation Systematique (ACURASYS) study and the Proning Severe ARDS Patients (PROSEVA) study.[7,41] In ACURASYS, 340 ARDS patients with a $PaO_2/FiO_2 \leq 150$ mm Hg were randomized to 48 hours of cisatracurium or placebo while heavily sedated early in ARDS.[41] Given the potential adverse effects of systemic paralysis and heavy sedation,[42,43] the study only enrolled patients with severe gas-exchange impairments

based on a $PaO_2/FiO_2 < 150$ mm Hg.[44] The early use of systemic cisatracurium significantly improved adjusted 90-day survival (hazard ratio [HR]: 0.68; 95% confidence interval [CI]: 0.48–0.98) and increased time off the ventilator without increasing muscle weakness. In PROSEVA, 466 ARDS patients also with a PaO_2/FiO_2 less than 150 mm Hg were randomized to undergo prone positioning for at least 16 hours a day or to be left supine. The PROSEVA investigators selected the 150 mm Hg cutoff based on prior meta-analyses of negative clinical trials that suggested a benefit to prone positioning only among those subjects severely hypoxemic at the time of enrollment.[45,46] Early prone positioning resulted in a reduction in 90-day mortality from 41.0 to 23.6% (HR: 0.44; 95% CI: 0.29–0.67). These two trials demonstrate the success of using a PaO_2/FiO_2 ratio to identify severe ARDS phenotypes for clinical trial enrollment, but this potential expands beyond the PaO_2/FiO_2 ratio. Several severity-scoring systems are used in clinical research, predict mortality, and may be useful to identify severity phenotypes of ARDS.[47–49] Additionally, while they are not included in the Berlin Definition, severity phenotypes based on respiratory parameters (e.g., lung compliance, dead space, or oxygenation ratio) also have the potential to provide useful information for future prognostic or predictive enrichment.[50–52]

Impact of Clinical Variables on ARDS Phenotypes

In the original description of ARDS, Ashbaugh and colleagues described a series of ARDS cases developing after the onset of heterogeneous precipitating illnesses.[1] The authors highlighted the similarity in the respiratory syndrome these patients developed despite distinct clinical characteristics; however, subsequent authors have recognized significant heterogeneity in ARDS based on several clinical variables. The first source of clinical heterogeneity described was the underlying precipitating factor for ARDS. The incidence of ARDS varies significantly across patients with different precipitating factors, ranging from 30 to 40% of sepsis patients to 10 to 25% of severe trauma patients.[53–57] In addition, the severity, biology, and outcomes vary as well. Sepsis-related ARDS is associated with a higher disease severity and higher mortality than nonsepsis-related ARDS, partially explained by the severity of illness as well as comorbidities.[58,59] Conversely, trauma-associated ARDS has been reported to be associated with a lower mortality than nontrauma-associated ARDS even after adjusting for baseline clinical factors and severity of illness.[60] In one study, patients with trauma-associated ARDS also had significantly lower plasma levels of markers of epithelial and endothelial injury but not markers of acute inflammation or disordered coagulation, suggesting biologic differences by ARDS precipitating risk factor.[60]

Another traditional way to subphenotype ARDS is by grouping patients into direct (or pulmonary) versus indirect (or extrapulmonary) ARDS.[3,61] The majority of direct lung injury results from pneumonia, aspiration, and pulmonary contusion, while the majority of indirect lung injury results from nonpulmonary sepsis, nonthoracic trauma, and transfusions.[62] This categorization is imperfect because many patients have mixed etiologies for lung injury and an overlap of direct and indirect insults[63]; however, several physiologic, pathologic, and biologic differences between direct and indirect ARDS have been reported. Pathologically, direct ARDS has been associated with significantly more alveolar collapse, fibrin deposition, and alveolar wall edema than indirect ARDS in an autopsy study.[64] Radiographically, direct ARDS has been characterized as a combination of ground glass opacities and consolidation with asymmetry of the consolidated areas.[65–67] Conversely, indirect ARDS has been characterized as predominantly ground glass opacities without significant consolidation and a more central distribution. Physiologically, patients with direct ARDS have been reported to have a higher lung elastance at baseline relative to indirect ARDS, but may be less likely to respond to PEEP.[67,68] There is also evidence that direct and indirect ARDS may differ biologically as well. Direct ARDS is associated with higher plasma concentrations of biomarkers of epithelial injury, including surfactant protein D (SP-D) and the receptor for advanced glycation end-products (RAGE).[69,70] In contrast, indirect ARDS is associated with higher plasma concentrations of biomarkers of endothelial injury, including angiopoietin-2 (Ang-2) and von Willebrand factor (VWF).[69,71–73] Additionally, several genetic polymorphisms have been reported to be associated with only direct or indirect ARDS but not the other subphenotype.[74] Despite the extensive research that has gone into characterizing direct and indirect ARDS, the two phenotypes overlap substantially, and their clinical utility is thus far limited.

The Berlin Definition requires ARDS to develop within 1 week of the onset of the underlying precipitating illness[4]; however, several researchers have identified significant heterogeneity based on the timing of onset of ARDS within that week.[75–78] Croce and colleagues first described early- and late-onset posttraumatic ARDS as distinct.[76] These authors defined early-onset ARDS as occurring within 48 hours after presentation and late-onset occurring beyond 48 hours after admission. Early-onset ARDS was associated with more profound hemorrhagic shock while pneumonia was significantly associated with late-onset ARDS. More recently, latent class analysis (LCA) was used to identify subphenotypes of posttraumatic ARDS based on the certainty of the ARDS diagnosis over time.[77] Similar to the earlier study, the 48-hour time point was identified to separate early-onset and late-onset phenotypes. The early-onset phenotype was again associated with hemorrhagic shock and the severity of thoracic trauma compared with the late-onset phenotype. Additionally, early-onset ARDS was also associated with higher plasma levels of RAGE and Ang-2, suggesting a biological distinction characterized by an alveolar capillary barrier injury pattern in this phenotype. Zhang and colleagues studied patients with moderate to severe ARDS precipitated by a variety of ARDS risk factors, again dividing patients between early- and late-onset ARDS at 48 hours after admission.[78] In this study, late-onset ARDS was associated with a higher mortality than early-onset ARDS despite lower severity of illness scores at admission.

One of the challenges in subphenotyping ARDS based on timing of ARDS onset is identifying the exact time of onset of the precipitating illness. In all of these studies, the onset time was the time of emergency department presentation or intensive care unit admission. In trauma, this time point is likely within several hours of traumatic injury, but in other precipitating syndromes such as sepsis, the exact beginning of the sepsis syndrome is not always clear. Additionally, variability may exist if the ARDS onset time is based on the time when all ARDS criteria are met (oxygenation and radiograph criteria) versus the time point when the first criterion is met.[79,80] Despite a wealth of studies describing subphenotypes of ARDS based on clinical factors, it is important to remember that no therapies specific to a clinical subphenotype have been proven effective. Conversely, data from the landmark ARDS Network ARMA trial suggests lung protective mechanical ventilation is effective in multiple clinical ARDS subtypes.[6]

Radiographic Phenotypes of ARDS

Radiographically, ARDS has largely been described as two phenotypes, nonfocal/diffuse ARDS and focal/lobar ARDS, based on morphologic characteristics on computed tomography (CT).[81] While there is significant correlation between these two categories and the clinical categories of direct and indirect lung injury, they do not fully overlap. ARDS categorized by diffuse rather than focal infiltrates has been associated with a higher mortality, lower lung compliance, more commonly indirect lung injury, and a lower inflection point on the pressure–volume curve of the lung.[82,83] Additionally, authors have reported distinct responses to the application of PEEP and recruitment maneuvers based on radiographic phenotype.[84,85] Patients with nonfocal or diffuse ARDS appear to have significant alveolar recruitment without significant overdistension with the application of increasing PEEP, while patients with focal ARDS tend to develop significant overdistension without recruitment. Supporting a biologic basis to radiographic phenotypes, another study reported a strong association of plasma concentrations of the epithelial biomarker RAGE and nonfocal CT based lung-imaging patterns in patients with ARDS.[83] A clinical trial is currently pending, which randomized patients to receive a traditional low tidal volume ventilation protocol versus a mechanical ventilation protocol tailored to radiographic lung morphology.[86]

Genetic and Biomarker-Defined Endotypes of ARDS

Approaches to phenotype ARDS based on clinical, radiologic, or severity variables are intuitive, particularly to clinicians who regularly treat ARDS; however, these approaches have only been modestly successful and fail to fully differentiate biologic differences that have the potential to be pharmacologic targets. Multiple biologic pathways have been implicated in ARDS, including endothelial and epithelial dysfunction, innate immune activation with immune cell recruitment, intravascular coagulation, and intraalveolar fibrosis.[87] However, the degree to which each pathogenic pathway is dominant in each individual patient is likely variable and based on a patient's clinical characteristics and genetics, as well as time from insult.[88] One approach to identify endotypes of ARDS is to classify patients based on genetic variability or based on the concentrations of biomarkers measured during critical illness that represent targetable biologic processes.

The heritability of ARDS is difficult to measure as the requirement for a major environmental insult precludes family pedigree studies; however, there is significant evidence that ARDS risk is altered by genetic variability.[74,88,89] Evolutionary pressures such as hemorrhagic shock, host–pathogen interactions, dehydration, and starvation select on mechanisms important in critical illness, including ARDS.[90] Therefore, it is reasonable to assume that evolutionary pressures which shape modern human genetic diversity impact ARDS risk and may be useful in identifying ARDS endotypes more likely to respond to novel therapeutics. Many discrete genetic polymorphisms in genes related to innate immunity, alveolar–capillary barrier function, surfactant function, oxidative stress, and other pathogenic pathways have been implicated in ARDS.[88,89] An extensive review of these genetic associations is beyond the scope of this article, but a few examples illustrate the potential impact of genetically defined endotypes on ARDS therapeutics.

One of the earliest reported genetic associations with ARDS is with a common deletion located in the human *ACE* gene that results in a higher plasma and tissue angiotensin converting enzyme (ACE) activity and a higher risk of ARDS.[91,92] The renin–angiotensin system has long been implicated in ARDS pathogenesis.[93] ACE is responsible for the conversion of angiotensin I to angiotensin II in the pulmonary vasculature, which results in vasoconstriction among other effects.[94] Therapeutics that negatively regulate the ACE axis, including ACE2, are currently being developed for ARDS.[95] A population that has a baseline higher ACE activity, such as one predicted by genetic variability in the *ACE* gene, might be an ideal population in which to test such a therapeutic.

Another relationship that has the potential to identify a genetically defined endotype is the association between ABO histo-blood type A and increased risk of ARDS.[96,97] The *ABO* gene encodes an enzyme responsible for placing terminal carbohydrate modifications on red blood cells as well as endothelial cells, epithelial cells, and platelets.[98] Variation in the *ABO* gene that determines ABO blood type is thought to have evolved via historic human–pathogen interactions, including with malaria.[99] The *ABO* gene is now recognized as among the most pleiotropic (one gene affecting multiple phenotypes) genes in the genome, and has been associated with multiple diseases and quantitative traits.[100–104] With regard to ARDS, *ABO* variation that determines blood type is strongly associated with plasma levels of multiple endothelial-derived glycoproteins important in ARDS, including VWF, intercellular adhesion molecule 1 (ICAM-1), and Ang-2.[104–108] It is possible that a therapeutic targeting these proteins or the endothelium in general could have a distinct

effect in different blood types, a genetic trait that is easily accessible clinically.

Similar to genetics, another approach to endotyping ARDS is to measure biomarkers representative of the activation of particular biologic pathways. These biomarkers may then be used to enroll patients in a clinical trial targeting their biology. Multiple plasma biomarkers have been reported to predict ARDS or ARDS outcome, including markers of inflammation (e.g., interleukin (IL)-6, IL-8),[109,110] endothelial activation and/or injury (e.g., Ang-2, ICAM-1, VWF),[111–113] epithelial injury (e.g., RAGE, SP-D),[114,115] and impaired coagulation (e.g., protein C).[116,117] One of the most promising plasma biomarkers for endotype identification in ARDS is Ang-2. Plasma Ang-2 is an established biomarker and mediator of endothelial activation and permeability and is strongly associated with ARDS risk and outcome.[72,111] Genetic variation in the Ang-2 gene (*ANGPT2*) is associated with ARDS risk,[118,119] and exogenous administration of Ang-2 potentiates lung injury in rodent models.[120] These data strongly suggest that Ang-2 causally contributes to ARDS pathogenesis; however, it is often difficult to distinguish biomarkers that causally contribute to disease and should be therapeutically targeted from biomarkers that only correlate with disease. For example, in cardiology the plasma low-density lipoprotein concentration causally contributes to heart disease and is therapeutically targeted while C-reactive protein predicts disease risk but does not causally contribute to heart disease.[121,122] One method to distinguish potentially causal from correlative biomarkers with observational data is mendelian randomization (MR) analysis.[123] In MR, an individual's genetic background is used as an instrumental variable to infer causality of a measured biomarker. In the case of Ang-2, MR analysis strongly suggests that plasma Ang-2 causally contributes to ARDS and should be prioritized for drug development.[124] It is possible that an endothelial endotype of ARDS defined by elevated plasma Ang-2 may be the group most likely to respond to Ang-2-targeted therapies.

Another plasma biomarker that may identify an endotype of ARDS is the IL-1 receptor antagonist (IL1RA). IL1RA is an inhibitory anti-inflammatory cytokine that competes with proinflammatory cytokines IL-1α and IL-1β to bind the IL-1 receptor without triggering receptor signaling.[125] In a large-scale genetic study, a coding genetic variant in the IL1RA gene (*IL1RN)* was associated with decreased risk of ARDS in multiple critically ill populations, as well as increased plasma IL1RA in the setting of sepsis and trauma.[126] These findings suggest that the coding variant in *IL1RN* confers protection from ARDS by increasing plasma IL1RA. Therefore, IL1RA has high potential for therapeutic benefit in ARDS. Three randomized placebo-controlled trials tested human recombinant IL1RA in sepsis, including patients with ARDS, but failed to demonstrate a benefit.[127–129] In a retrospective secondary analysis of one of these trials, Meyer et al demonstrated significant heterogeneity of treatment effect based on plasma concentrations of IL1RA measured at study enrollment.[130] Patients with a higher endogenous IL1RA had a statistically significant survival benefit to recombinant IL1RA therapy, while those with lower IL1RA did not. The test for interaction was also statistically significant, suggesting a true difference in the treatment effect between groups stratified by measured endogenous IL1RA. While it may be counterintuitive that patients with high endogenous IL1RA also had a benefit to the administration of recombinant IL1RA, endogenous IL1RA may be serving as a biomarker of activation of the entire IL-1 axis. In the study conducted by Meyer et al, IL1RA was more easily measured than the proinflammatory marker of the IL-1 axis, IL-1β. Future precision trials of recombinant IL1RA for an IL-1 axis endotype of ARDS warrant further consideration. Other promising endotype-defining biomarkers that could be targeted therapeutically include soluble RAGE (sRAGE), thrombomodulin, and tumor necrosis factor receptor-1 (TNFR-1), among others.[114,131–133]

Unbiased Approaches to Endotype ARDS

As our understanding of the complex biology of ARDS has improved, mathematical and statistical methods to understand heterogeneity have also advanced. Cluster analysis techniques, such as hierarchical or k-means clustering, have been used to identify groups within a larger population with similar characteristics. As discussed previously, these methods have been used successfully in asthma to identify subphenotypes with distinguishable clinical characteristics and response to asthma therapies.[21,22] In critical care, Wong and colleagues used cluster-based techniques to identify two subphenotypes of pediatric sepsis with distinct outcomes using whole blood gene expression data.[134,135] The two subphenotypes were subsequently found to have distinct responses to systemic corticosteroids.[136] Similarly, in adult sepsis populations, researchers have identified two sepsis response signatures in peripheral blood leukocyte gene expression data using hierarchical clustering.[137,138] The first sepsis response signature was characterized by an immunosuppressed phenotype and was associated with a higher mortality than the second sepsis response signature. Another group of investigators used similar methods to identify four phenotypes of sepsis with significant overlap with the two sepsis response signatures.[139] Cluster analyses have advantages including an unbiased mathematical approach to the data based on characteristics introduced to the model rather than preexisting assumptions. This approach allows for the discovery of biologically significant endotypes that may not be readily apparent. However, cluster-based approaches are limited in that they only identify heterogeneity based on the variables considered and can suffer from problems with overfit data and challenges with replication.[140]

Another method to endotype syndromes, used with some success in ARDS and similar to cluster analysis, is LCA. While cluster analysis is a mathematical method to identify clusters, LCA is a statistical modeling method that identifies unobserved (latent) groups within a heterogeneous population.[141] In ARDS, LCA has been used to identify two distinct subphenotypes characterized by distinct biology, outcomes, and response to therapy.[142–144] Initially using data from patients enrolled in the NHLBI ARDSNet ARMA and ALVEOLI trials,[6,145] LCA was applied to clinical and plasma biomarker data and identified a hyperinflammatory phenotype of ARDS.[142] This phenotype was characterized by higher

plasma concentrations of inflammatory biomarkers, worse shock, and higher mortality. Additionally, the effect of treatment with PEEP differed based on phenotype whereby the high PEEP strategy appeared to be more effective in the hyperinflamed phenotype. The identified subphenotypes were subsequently reported to be largely stable over the first 3 days of trial enrollment,[143] and were validated in a post-hoc analysis of the FACTT trial.[144,146] Additionally, the ARDS subphenotypes responded differently to the fluid liberal versus conservative strategy tested in the FACTT trial. Specifically, the hyperinflamed subtype had a higher mortality with a conservative fluid strategy while the other subtype had a lower mortality with a conservative fluid strategy. These findings again suggest heterogeneity in the response to therapies that have previously been applied in a "one size fits all" paradigm. In this study, the authors also developed a simple model based on three biomarkers (IL-8, TNFR1, and bicarbonate) to categorize patients accurately in the two subphenotypes. If validated prospectively, this simpler model may remove the need to re-perform the complicated LCA analysis to phenotype ARDS patients making endotype identification potentially possible clinically.

LCA has also been applied to data from pharmacologic trials, specifically those studying the efficacy of statins for ARDS.[147,148] In secondary analyses of both the HARP-2 trial investigating simvastatin and the SAILS trial investigating rosuvastatin,[149,150] LCA identified two subphenotypes of ARDS with similar biological characteristics, outcomes, and distributions as the prior trials.[147,148] While the HARP-2 and the SAILS trials were negative, the hyperinflammatory subphenotype of ARDS had improved survival with simvastatin compared with placebo. This survival benefit was not seen with rosuvastatin in SAILS, for reasons that may be related to differences in the two statins' biology, dosing, clinical trial design, or patient populations. Despite identifying benefits to PEEP, fluid strategy, and simvastatin specific to LCA-defined endotypes, these analyses are post hoc and should not be implemented without prospective trials.

Other investigators have also applied clustering techniques to an observational cohort study of patients with ARDS.[151] In one study, cluster analysis was performed using 20 plasma biomarkers of inflammation, coagulation, and endothelial activation, without any clinical data. Again, two subphenotypes of ARDS were identified and described as uninflamed and reactive. The reactive phenotype was associated with a higher mortality and could be accurately identified using five biomarkers (IL-6, interferon gamma, angiopoietin 1/2, and plasminogen activator inhibitor-1). These authors subsequently demonstrated that a third of genes are differentially expressed in whole blood between the two subphenotypes, providing further evidence of significant biological heterogeneity.[152] These identified subphenotypes share similar characteristics to those identified in the LCA analyses conducted by Calfee and colleagues, though the precise degree of overlap between the two approaches remains unknown. ►**Table 2** includes a comparison of the subphenotypes of ARDS identified via LCA or cluster analysis.

Implications for Clinical Trials

Given the number of failed past trials, it is clear that our design of clinical trials in ARDS needs to evolve, and the preceding evidence suggests that incorporating endotypes and/or subtypes of disease into clinical trials in ARDS may offer an improved approach. Prognostic enrichment based on the severity of oxygenation impairment has demonstrated some success[7,41]; however, the true potential of precision medicine rests on predictive enrichment based on biologically defined phenotypes or endotypes. Endotypes defined by one or multiple biomarkers and/or clinical characteristics have the

Table 2 Unbiased approaches to identify ARDS endotypes applied to populations from five clinical trials and one cohort study

Cohort	Phenotype	% of study population	90-day mortality	Heterogeneity of treatment response	Reference
ARMA	• Hypoinflammatory • Hyperinflammatory	67% 33%	23% 44%		142
ALVEOLI	• Hypoinflammatory • Hyperinflammatory	74% 26%	19% 51%	Differences in response to a high PEEP strategy	142
FACTT	• Hypoinflammatory • Hyperinflammatory	73% 27%	22% 45%	Differences in response to a liberal vs. conservative fluid strategy	144
MARS	• Uninflamed • Reactive	48% 52%	22%[a] 38%[a]		151
HARP-2	• Hypoinflammatory • Hyperinflammatory	65% 35%	17% 39%	Differences in response to simvastatin therapy	148
SAILS	• Hypoinflammatory • Hyperinflammatory	60% 40%	21% 38%	No differences based on rosuvastatin	147

Abbreviations: ALVEOLI, Assessment of Low Tidal Volume and Elevated End-expiratory volume to Obviate Lung Injury Study; ARDS, acute respiratory distress syndrome; ARMA, Prospective, Randomized, Multicenter Trial of 12 vs. 6 mL/kg Tidal Volume Positive Pressure Ventilation for Treatment of Acute Lung Injury and Acute Respiratory Distress Syndrome; FACTT, Fluid and Catheter Treatment Trial; HARP-2, HMG-CoA Reductase Inhibition in Acute Lung Injury to Reduce Pulmonary Dysfunction Trial; MARS, Molecular Diagnosis and Risk Stratification for Sepsis Cohort Study; PEEP, positive end expiratory pressure; SAILS, Statins for Acutely Injured Lungs from Sepsis Study.
[a]30-day mortality.

potential to select patients most likely to benefit from new pharmacologic therapies targeting specific biologic pathways. Clinical trial enrollment could start by measuring a specific plasma marker and only including those subjects over a prespecified threshold concentration. Alternatively, innovative adaptive trial designs could enroll all patients regardless of biomarker concentration, but subsequently adjust randomization so patients not benefiting from a therapy based on their biomarker level are less likely to be randomized to the therapy within a clinical trial.[153]

Several challenges exist before biomarker-driven clinical trials for ARDS can be initiated. First, biomarkers must be rapidly measurable to be of utility in critical care. The technology to measure protein biomarkers or gene expression rapidly is available; however, few tests are currently available clinically. Second, biomarker-driven trials require more knowledge of the performance, stability, and responsiveness of the biomarker over time as patients progress to ARDS. Third, thresholds of biomarker levels by which patients may benefit from a therapy are largely unclear and may require further prospective testing or initial enrollment of all patients in an adaptive clinical trial. Fourth, we have limited mechanistic understanding of ARDS endotypes and must have strong evidence that a target drug works only in one subtype prior to initiating a targeted clinical trial.

Conclusions

In conclusion, ARDS is a heterogeneous syndrome with a lack of therapies directed at syndrome biology. In the last several years, distinct subphenotypes of ARDS have been described with distinct clinical, pathologic, radiographic, and/or biologic characteristics. Early evidence of differential response to therapies based on subtypes defined by plasma biomarkers and clinical characteristics has been reported. Future work should focus on furthering our mechanistic understanding of ARDS endotypes, identifying more important ARDS phenotypes, and developing targeted therapies with the ultimate goal of applying these therapies to the patients most likely to respond to them.

Conflict of Interest

Dr. Calfee reports grants from NIH, grants and personal fees from GlaxoSmithKline, grants and personal fees from Bayer, personal fees from CSL Behring, personal fees from Prometic, personal fees from Roche/Genentech, personal fees from Quark, outside the submitted work. Dr. Christie reports grants from NIH, during the conduct of the study; grants from NIH, grants from GlaxoSmithKline, and grants from Bristol Meyers Squibb outside the submitted work; and has served as an advisory board member for Onspira Therapeutics. Dr. Reilly reports grants from National Institutes of Health, during the conduct of the study.

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